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## (54) Fem A gene of staphylococcus epidermidis, fem A protein, and vectors of microorganisms comprising the fem A gene.

(57) The instant invention provides the *femA* gene of *Staphylococcus epidermidis* and all degenerate sequences thereof, the protein encoded by the *femA* gene (FemA), and vectors and microorganisms comprising genes encoding the FemA protein.

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Clinical isolates of staphylococci (*Staphylococcus aureus and S. epidermidis*) which cause serious infections due to their intrinsic resistance to beta-lactamase-stable beta-lactam antibiotics (*e.g.,* methicillin) carry the *mecA* gene. Song et al., *FEBS Lett.* **221**:167-171 (1987). This gene encodes a putative cell wall biosynthetic enzyme referred to as penicillin binding protein 2a (PBP2a). PBP2a, which binds beta-lactams only at concentrations well above therapeutic efficacy, apparently can functionally substitute for all the staphylococcal PBPs and permit growth when the host organism is threatened by beta-lactams. Hartman and Tomasz, *J. Bacteriol.* **158**:513-516 (1984). Wu et al., *Antimicrob. Agents Chemother.* **36**:533-539 (1992) and Ryffel et al., *Gene* **94**:137-138 (1990).

The *mecA* gene is not a normal part of the staphylococcal genome. The organism which donated *mecA* to the staphylococci remains unidentified. Despite the uniform presence of *mecA* in methicillin-resistant clinical isolates, these isolates vary considerably in their degree of resistance to methicillin. This variation in phenotypic expression within a population has been referred to as heterogenous expression. Matthews and Stewart, *FEMS Microbiol. Lett.* **22**:161-166 (1984). Typically, most cells exhibit low-level resistance to methicillin and only a minority of the population express high-level resistance, perhaps only one in $10^8$ cells. Tomasz et al., *Antimicrob. Agents Chemother.* **35**:124-129 (1991). Although expression of methicillin resistance is dependent upon the presence of FBP2a, it appears to be somewhat independent of the amount of PBP2a, suggesting important roles for other factors. Chambers and Hackbarth, *Antimicrob. Agents Chemother.* **31**:1982-1988 (1987) and Murakami and Tomasz, *J. Bacteriol.* **171**:874-879 (1989).

Tn*551* insertional mutagenesis of methicillin-resistant *S. aureus* revealed numerous sites which influence the level of methicillin resistance but are not linked to *mecA* and do not perturb the expression of PBP2a. Berger-Bächi et al., *Antimicrob. Agents Chemother.* **36**:1367-1373 (1992); Kornblum et al., *Eur. J. Clin. Microbiol.* **5**:714-718 (1986); Berger-Bächi et al., *Mol. Gen. Genet.* **219**:263-269 (1989) and Maidhof et al., *J. Bacteriol.* **173**:3507-3513 (1991). Those factors described thus far generally depress the MIC of beta-lactam resistant strains. Some of the genetic loci which demonstrate such an effect on methicillin resistance were designated factors essential for methicillin resistance (*fem*). Berger-Bächi et al., *Mol. Gen. Genet.* **219**:263-269 (1989). In contrast to *mecA,* the genes which encode influential factors are probably present in both resistant and susceptible strains of *S. aureus* and *S. epidermidis.* Information obtained from gene disruption studies of *femA* and *femB* in *S. aureus* indicated that in addition to enhanced sensitivity to methicillin, homogeneously methicillin-resistant *S. aureus* strains carrying such gene disruptions have a reduced glycine content in the peptidoglycan component of their cell walls (Maidhof et al., *J. Bacteriol.* **173**:3507-3513 (1991)) and exhibit reduced rates of cell wall turnover and autolysis (de Jonge et al., *J. Bacteriol.* **173**:1105-1110 (1991)).

Genetic factors, other than *mecA,* that influence the expression of methicillin resistance in *S. epidermidis* have, until now, not been described at the molecular level. The present invention provides DNA sequences encoding the FemA protein of *Staphylococcus epidermidis,* the FemA protein itself, and vectors and microorganisms comprising the *femA* gene of *S. epidermidis.*

The present invention provides DNA sequences encoding the FemA protein of *Staphylococcus epidermidis,* including the natural gene sequence designated *femA* (SEQ ID NO:1). Thus, included in the present invention is any DNA compound that comprises an isolated DNA sequence encoding SEQ ID NO:2. SEQ ID NO:2 is as follows:

```
Met Lys Met Lys Phe Thr Asn Leu Thr Ala Lys Glu Phe Ser Asp Phe
1               5                  10                  15

Thr Asp Arg Met Thr Tyr Ser His Phe Thr Gln Met Glu Gly Asn Tyr
            20                  25                  30

Glu Leu Lys Val Ala Glu Gly Thr Glu Ser His Leu Val Gly Ile Lys
        35                  40                  45

Asn Asn Asp Asn Glu Val Ile Ala Ala Cys Leu Leu Thr Ala Val Pro
    50                  55                  60

Val Met Lys Ile Phe Lys Tyr Phe Tyr Ser Asn Arg Gly Pro Val Ile
65                  70                  75                  80

Asp Tyr Asn Asn Lys Glu Leu Val His Phe Phe Phe Asn Glu Leu Ser
                85                  90                  95

Lys Tyr Val Lys Lys Tyr Asn Cys Leu Tyr Leu Arg Val Asp Pro Tyr
            100                 105                 110

Leu Pro Tyr Gln Tyr Leu Asn His Glu Gly Glu Ile Thr Gly Asn Ala
        115                 120                 125

Gly His Asp Trp Ile Phe Asp Glu Leu Glu Ser Leu Gly Tyr Lys His
    130                 135                 140

Glu Gly Phe His Lys Gly Phe Asp Pro Val Leu Gln Ile Arg Tyr His
145                 150                 155                 160

Ser Val Leu Asn Leu Ala Asn Lys Ser Ala Asn Asp Val Leu Lys Asn
                165                 170                 175

Met Asp Gly Leu Arg Lys Arg Asn Thr Lys Lys Val Lys Lys Asn Gly
            180                 185                 190

Val Lys Val Arg Phe Leu Ser Glu Glu Glu Leu Pro Ile Phe Arg Ser
        195                 200                 205

Phe Met Glu Asp Thr Ser Glu Thr Lys Asp Phe Ala Asp Arg Glu Asp
    210                 215                 220

Ser Phe Tyr Tyr Asn Arg Phe Lys His Tyr Lys Asp Arg Val Leu Val
225                 230                 235                 240
```

```
Pro Leu Ala Tyr Ile Asn Phe Asp Glu Tyr Ile Glu Glu Leu Asn Asn
                245                 250                 255

Glu Arg Asn Val Leu Asn Lys Asp Tyr Asn Lys Ala Leu Lys Asp Ile
            260                 265                 270

Glu Lys Arg Pro Glu Asn Lys Lys Ala His Asn Lys Lys Glu Asn Leu
        275                 280                 285

Glu Gln Gln Leu Asp Ala Asn Gln Gln Lys Ile Asn Glu Ala Lys Asn
    290                 295                 300

Leu Lys Gln Glu His Gly Asn Glu Leu Pro Ile Ser Ala Gly Phe Phe
305                 310                 315                 320

Ile Ile Asn Pro Phe Glu Val Val Tyr Tyr Ala Gly Gly Thr Ser Asn
                325                 330                 335

Arg Tyr Arg His Phe Ala Gly Ser Tyr Ala Val Gln Trp Lys Met Ile
            340                 345                 350

Asn Tyr Ala Ile Glu His Gly Ile Asn Arg Tyr Asn Phe Tyr Gly Ile
        355                 360                 365

Ser Gly Asp Phe Ser Glu Asp Ala Glu Asp Ala Gly Val Val Lys Phe
    370                 375                 380

Lys Lys Gly Tyr Asp Ala Asp Val Ile Glu Tyr Val Gly Asp Phe Ile
385                 390                 395                 400

Lys Pro Ile Asn Lys Pro Met Tyr Asn Ile Tyr Arg Thr Leu Lys Lys
                405                 410                 415

Leu Lys Lys
```

The natural *femA* sequence is encompassed by the present invention as a DNA compound which comprises the isolated DNA sequence which is SEQ ID NO:1. SEQ ID NO:1 is as follows:

```
ATG AAG ATG AAG TTT ACG AAT TTG ACA GCT AAA GAA TTT AGT GAC TTT 48
Met Lys Met Lys Phe Thr Asn Leu Thr Ala Lys Glu Phe Ser Asp Phe
1               5                   10                  15

ACT GAT CGT ATG ACA TAT AGT CAT TTT ACA CAA ATG GAA GGT AAT TAC 96
Thr Asp Arg Met Thr Tyr Ser His Phe Thr Gln Met Glu Gly Asn Tyr
            20                  25                  30
```

```
GAA TTA AAG GTT GCT GAA GGT ACC GAG TCA CAT TTA GTT GGA ATT AAA 144
Glu Leu Lys Val Ala Glu Gly Thr Glu Ser His Leu Val Gly Ile Lys
        35                  40                  45

AAT AAT GAT AAC GAA GTG ATT GCA GCT TGT TTA TTA ACA GCT GTT CCT 192
Asn Asn Asp Asn Glu Val Ile Ala Ala Cys Leu Leu Thr Ala Val Pro
    50                  55                  60

GTA ATG AAA ATA TTT AAA TAT TTT TAT TCC AAT CGC GGT CCA GTA ATA 240
Val Met Lys Ile Phe Lys Tyr Phe Tyr Ser Asn Arg Gly Pro Val Ile
65                  70                  75                  80

GAT TAT AAT AAT AAA GAG CTT GTA CAT TTT TTC TTT AAT GAA TTG AGT 288
Asp Tyr Asn Asn Lys Glu Leu Val His Phe Phe Phe Asn Glu Leu Ser
                85                  90                  95

AAA TAT GTA AAA AAA TAT AAT TGT TTA TAT TTA AGA GTT GAC CCA TAC 336
Lys Tyr Val Lys Lys Tyr Asn Cys Leu Tyr Leu Arg Val Asp Pro Tyr
            100                 105                 110

CTT CCA TAT CAA TAT TTA AAT CAT GAG GGA GAA ATA ACT GGA AAT GCA 384
Leu Pro Tyr Gln Tyr Leu Asn His Glu Gly Glu Ile Thr Gly Asn Ala
        115                 120                 125

GGT CAT GAT TGG ATT TTT GAT GAA TTA GAG AGT TTA GGA TAT AAA CAC 432
Gly His Asp Trp Ile Phe Asp Glu Leu Glu Ser Leu Gly Tyr Lys His
    130                 135                 140

GAA GGA TTC CAC AAA GGA TTT GAT CCT GTA TTA CAA ATC CGA TAT CAT 480
Glu Gly Phe His Lys Gly Phe Asp Pro Val Leu Gln Ile Arg Tyr His
145                 150                 155                 160

TCT GTT CTA AAT TTA GCA AAC AAA AGT GCT AAT GAT GTT TTA AAA AAC 528
Ser Val Leu Asn Leu Ala Asn Lys Ser Ala Asn Asp Val Leu Lys Asn
                165                 170                 175

ATG GAT GGT TTA AGA AAG CGT AAT ACT AAA AAA GTT AAG AAA AAT GGA 576
Met Asp Gly Leu Arg Lys Arg Asn Thr Lys Lys Val Lys Lys Asn Gly
            180                 185                 190

GTT AAA GTC CGC TTT TTA TCT GAA GAA GAG TTA CCT ATA TTT AGG TCA 624
Val Lys Val Arg Phe Leu Ser Glu Glu Glu Leu Pro Ile Phe Arg Ser
        195                 200                 205

TTT ATG GAG GAT ACC TCT GAA ACT AAA GAT TTT GCA GAT AGA GAA GAT 672
Phe Met Glu Asp Thr Ser Glu Thr Lys Asp Phe Ala Asp Arg Glu Asp
    210                 215                 220

AGT TTT TAT TAC AAC AGA TTC AAA CAT TAT AAA GAC CGT GTT TTA GTA 720
Ser Phe Tyr Tyr Asn Arg Phe Lys His Tyr Lys Asp Arg Val Leu Val
225                 230                 235                 240

CCA CTA GCC TAT ATT AAC TTT GAT GAG TAT ATA GAG GAA CTA AAT AAT 768
Pro Leu Ala Tyr Ile Asn Phe Asp Glu Tyr Ile Glu Glu Leu Asn Asn
                245                 250                 255
```

```
GAA AGA AAT GTG CTT AAT AAA GAT TAT AAT AAA GCT TTA AAA GAC ATT 816
Glu Arg Asn Val Leu Asn Lys Asp Tyr Asn Lys Ala Leu Lys Asp Ile
            260                 265                 270

GAG AAA CGT CCA GAG AAT AAA AAA GCA CAT AAC AAA AAG GAA AAT TTA 864
Glu Lys Arg Pro Glu Asn Lys Lys Ala His Asn Lys Lys Glu Asn Leu
        275                 280                 285

GAA CAA CAA CTC GAT GCA AAT CAG CAA AAA ATT AAT GAA GCT AAA AAC 912
Glu Gln Gln Leu Asp Ala Asn Gln Gln Lys Ile Asn Glu Ala Lys Asn
    290                 295                 300

TTA AAA CAA GAA CAT GGC AAT GAA TTA CCC ATC TCT GCT GGC TTC TTT 960
Leu Lys Gln Glu His Gly Asn Glu Leu Pro Ile Ser Ala Gly Phe Phe
305                 310                 315                 320

ATA ATT AAT CCG TTT GAA GTA GTT TAC TAC GCT GGT GGA ACT TCA AAT 1008
Ile Ile Asn Pro Phe Glu Val Val Tyr Tyr Ala Gly Gly Thr Ser Asn
                325                 330                 335

CGT TAT CGC CAT TTT GCA GGG AGC TAT GCG GTT CAA TGG AAG ATG ATT 1056
Arg Tyr Arg His Phe Ala Gly Ser Tyr Ala Val Gln Trp Lys Met Ile
            340                 345                 350

AAC TAT GCA ATT GAA CAT GGT ATT AAT CGG TAT AAT TTC TAT GGT ATT 1104
Asn Tyr Ala Ile Glu His Gly Ile Asn Arg Tyr Asn Phe Tyr Gly Ile
        355                 360                 365

AGT GGT GAC TTT AGT GAA GAT GCT GAA GAT GCT GGC GTA GTT AAG TTT 1152
Ser Gly Asp Phe Ser Glu Asp Ala Glu Asp Ala Gly Val Val Lys Phe
    370                 375                 380

AAA AAG GGC TAT GAT GCC GAT GTT ATA GAA TAC GTT GGT GAC TTT ATT 1200
Lys Lys Gly Tyr Asp Ala Asp Val Ile Glu Tyr Val Gly Asp Phe Ile
385                 390                 395                 400

AAA CCT ATT AAT AAA CCA ATG TAT AAC ATT TAT AGA ACA CTT AAA AAA 1248
Lys Pro Ile Asn Lys Pro Met Tyr Asn Ile Tyr Arg Thr Leu Lys Lys
                405                 410                 415

CTA AAG AAA 1257
Leu Lys Lys
```

The present invention also includes the protein encoded by SEQ ID NO: 1 in purified form. Also included are recombinant DNA vectors, including expression vectors, that comprise DNA sequences encoding FemA.

The restriction site and function maps presented in the accompanying drawings are approximate representations of the recombinant DNA vectors discussed herein. The restriction site information is not exhaustive; therefore, there may be more restriction sites of a given type on the vector than actually shown on the map.

Figure 1 - A restriction site and function map of plasmid pPSJ180.

Figure 2 - A restriction site and function map of plasmid pET-11A.

The instant invention provides the *femA* gene of *Staphylococcus epidermidis* and all degenerate sequences thereof, the protein encoded by the *femA* gene (FemA), and vectors and microorganisms comprising genes encoding the FemA protein. In the practice of the invention as exemplified herein, the FemA protein comprises the amino acid sequence, which is SEQ ID NO 2:

```
Met Lys Met Lys Phe Thr Asn Leu Thr Ala Lys Glu Phe Ser Asp Phe
1               5                  10                  15

Thr Asp Arg Met Thr Tyr Ser His Phe Thr Gln Met Glu Gly Asn Tyr
            20                  25                  30

Glu Leu Lys Val Ala Glu Gly Thr Glu Ser His Leu Val Gly Ile Lys
        35                  40                  45

Asn Asn Asp Asn Glu Val Ile Ala Ala Cys Leu Leu Thr Ala Val Pro
    50                  55                  60

Val Met Lys Ile Phe Lys Tyr Phe Tyr Ser Asn Arg Gly Pro Val Ile
65                  70                  75                  80

Asp Tyr Asn Asn Lys Glu Leu Val His Phe Phe Phe Asn Glu Leu Ser
                85                  90                  95

Lys Tyr Val Lys Lys Tyr Asn Cys Leu Tyr Leu Arg Val Asp Pro Tyr
            100                 105                 110

Leu Pro Tyr Gln Tyr Leu Asn His Glu Gly Glu Ile Thr Gly Asn Ala
        115                 120                 125

Gly His Asp Trp Ile Phe Asp Glu Leu Glu Ser Leu Gly Tyr Lys His
    130                 135                 140

Glu Gly Phe His Lys Gly Phe Asp Pro Val Leu Gln Ile Arg Tyr His
145                 150                 155                 160
```

```
Ser Val Leu Asn Leu Ala Asn Lys Ser Ala Asn Asp Val Leu Lys Asn
                165                 170                 175

Met Asp Gly Leu Arg Lys Arg Asn Thr Lys Lys Val Lys Lys Asn Gly
            180                 185                 190

Val Lys Val Arg Phe Leu Ser Glu Glu Glu Leu Pro Ile Phe Arg Ser
        195                 200                 205

Phe Met Glu Asp Thr Ser Glu Thr Lys Asp Phe Ala Asp Arg Glu Asp
    210                 215                 220

Ser Phe Tyr Tyr Asn Arg Phe Lys His Tyr Lys Asp Arg Val Leu Val
225                 230                 235                 240

Pro Leu Ala Tyr Ile Asn Phe Asp Glu Tyr Ile Glu Glu Leu Asn Asn
                245                 250                 255

Glu Arg Asn Val Leu Asn Lys Asp Tyr Asn Lys Ala Leu Lys Asp Ile
            260                 265                 270

Glu Lys Arg Pro Glu Asn Lys Lys Ala His Asn Lys Lys Glu Asn Leu
        275                 280                 285

Glu Gln Gln Leu Asp Ala Asn Gln Gln Lys Ile Asn Glu Ala Lys Asn
    290                 295                 300

Leu Lys Gln Glu His Gly Asn Glu Leu Pro Ile Ser Ala Gly Phe Phe
305                 310                 315                 320

Ile Ile Asn Pro Phe Glu Val Val Tyr Tyr Ala Gly Gly Thr Ser Asn
                325                 330                 335

Arg Tyr Arg His Phe Ala Gly Ser Tyr Ala Val Gln Trp Lys Met Ile
            340                 345                 350

Asn Tyr Ala Ile Glu His Gly Ile Asn Arg Tyr Asn Phe Tyr Gly Ile
        355                 360                 365

Ser Gly Asp Phe Ser Glu Asp Ala Glu Asp Ala Gly Val Val Lys Phe
    370                 375                 380

Lys Lys Gly Tyr Asp Ala Asp Val Ile Glu Tyr Val Gly Asp Phe Ile
385                 390                 395                 400

Lys Pro Ile Asn Lys Pro Met Tyr Asn Ile Tyr Arg Thr Leu Lys Lys
                405                 410                 415

Leu Lys Lys
```

The present invention also provides the natural *femA* gene found in *Staphylococcus epidermidis*, embodied in SEQ ID NO: 1:

```
ATG AAG ATG AAG TTT ACG AAT TTG ACA GCT AAA GAA TTT AGT GAC TTT 48
Met Lys Met Lys Phe Thr Asn Leu Thr Ala Lys Glu Phe Ser Asp Phe
1               5                  10                  15

ACT GAT CGT ATG ACA TAT AGT CAT TTT ACA CAA ATG GAA GGT AAT TAC 96
Thr Asp Arg Met Thr Tyr Ser His Phe Thr Gln Met Glu Gly Asn Tyr
            20                  25                  30

GAA TTA AAG GTT GCT GAA GGT ACC GAG TCA CAT TTA GTT GGA ATT AAA 144
Glu Leu Lys Val Ala Glu Gly Thr Glu Ser His Leu Val Gly Ile Lys
        35                  40                  45

AAT AAT GAT AAC GAA GTG ATT GCA GCT TGT TTA TTA ACA GCT GTT CCT 192
Asn Asn Asp Asn Glu Val Ile Ala Ala Cys Leu Leu Thr Ala Val Pro
    50                  55                  60

GTA ATG AAA ATA TTT AAA TAT TTT TAT TCC AAT CGC GGT CCA GTA ATA 240
Val Met Lys Ile Phe Lys Tyr Phe Tyr Ser Asn Arg Gly Pro Val Ile
65                  70                  75                  80

GAT TAT AAT AAT AAA GAG CTT GTA CAT TTT TTC TTT AAT GAA TTG AGT 288
Asp Tyr Asn Asn Lys Glu Leu Val His Phe Phe Phe Asn Glu Leu Ser
                85                  90                  95

AAA TAT GTA AAA AAA TAT AAT TGT TTA TAT TTA AGA GTT GAC CCA TAC 336
Lys Tyr Val Lys Lys Tyr Asn Cys Leu Tyr Leu Arg Val Asp Pro Tyr
            100                 105                 110

CTT CCA TAT CAA TAT TTA AAT CAT GAG GGA GAA ATA ACT GGA AAT GCA 384
Leu Pro Tyr Gln Tyr Leu Asn His Glu Gly Glu Ile Thr Gly Asn Ala
        115                 120                 125

GGT CAT GAT TGG ATT TTT GAT GAA TTA GAG AGT TTA GGA TAT AAA CAC 432
Gly His Asp Trp Ile Phe Asp Glu Leu Glu Ser Leu Gly Tyr Lys His
    130                 135                 140

GAA GGA TTC CAC AAA GGA TTT GAT CCT GTA TTA CAA ATC CGA TAT CAT 480
Glu Gly Phe His Lys Gly Phe Asp Pro Val Leu Gln Ile Arg Tyr His
145                 150                 155                 160

TCT GTT CTA AAT TTA GCA AAC AAA AGT GCT AAT GAT GTT TTA AAA AAC 528
Ser Val Leu Asn Leu Ala Asn Lys Ser Ala Asn Asp Val Leu Lys Asn
                165                 170                 175

ATG GAT GGT TTA AGA AAG CGT AAT ACT AAA AAA GTT AAG AAA AAT GGA 576
Met Asp Gly Leu Arg Lys Arg Asn Thr Lys Lys Val Lys Lys Asn Gly
            180                 185                 190
```

```
GTT AAA GTC CGC TTT TTA TCT GAA GAA GAG TTA CCT ATA TTT AGG TCA 624
Val Lys Val Arg Phe Leu Ser Glu Glu Glu Leu Pro Ile Phe Arg Ser
        195                 200                 205

TTT ATG GAG GAT ACC TCT GAA ACT AAA GAT TTT GCA GAT AGA GAA GAT 672
Phe Met Glu Asp Thr Ser Glu Thr Lys Asp Phe Ala Asp Arg Glu Asp
    210                 215                 220

AGT TTT TAT TAC AAC AGA TTC AAA CAT TAT AAA GAC CGT GTT TTA GTA 720
Ser Phe Tyr Tyr Asn Arg Phe Lys His Tyr Lys Asp Arg Val Leu Val
225                 230                 235                 240

CCA CTA GCC TAT ATT AAC TTT GAT GAG TAT ATA GAG GAA CTA AAT AAT 768
Pro Leu Ala Tyr Ile Asn Phe Asp Glu Tyr Ile Glu Glu Leu Asn Asn
                245                 250                 255

GAA AGA AAT GTG CTT AAT AAA GAT TAT AAT AAA GCT TTA AAA GAC ATT 816
Glu Arg Asn Val Leu Asn Lys Asp Tyr Asn Lys Ala Leu Lys Asp Ile
            260                 265                 270

GAG AAA CGT CCA GAG AAT AAA AAA GCA CAT AAC AAA AAG GAA AAT TTA 864
Glu Lys Arg Pro Glu Asn Lys Lys Ala His Asn Lys Lys Glu Asn Leu
        275                 280                 285

GAA CAA CAA CTC GAT GCA AAT CAG CAA AAA ATT AAT GAA GCT AAA AAC 912
Glu Gln Gln Leu Asp Ala Asn Gln Gln Lys Ile Asn Glu Ala Lys Asn
    290                 295                 300

TTA AAA CAA GAA CAT GGC AAT GAA TTA CCC ATC TCT GCT GGC TTC TTT 960
Leu Lys Gln Glu His Gly Asn Glu Leu Pro Ile Ser Ala Gly Phe Phe
305                 310                 315                 320

ATA ATT AAT CCG TTT GAA GTA GTT TAC TAC GCT GGT GGA ACT TCA AAT 1008
Ile Ile Asn Pro Phe Glu Val Val Tyr Tyr Ala Gly Gly Thr Ser Asn
                325                 330                 335

CGT TAT CGC CAT TTT GCA GGG AGC TAT GCG GTT CAA TGG AAG ATG ATT 1056
Arg Tyr Arg His Phe Ala Gly Ser Tyr Ala Val Gln Trp Lys Met Ile
            340                 345                 350

AAC TAT GCA ATT GAA CAT GGT ATT AAT CGG TAT AAT TTC TAT GGT ATT 1104
Asn Tyr Ala Ile Glu His Gly Ile Asn Arg Tyr Asn Phe Tyr Gly Ile
        355                 360                 365

AGT GGT GAC TTT AGT GAA GAT GCT GAA GAT GCT GGC GTA GTT AAG TTT 1152
Ser Gly Asp Phe Ser Glu Asp Ala Glu Asp Ala Gly Val Val Lys Phe
    370                 375                 380

AAA AAG GGC TAT GAT GCC GAT GTT ATA GAA TAC GTT GGT GAC TTT ATT 1200
Lys Lys Gly Tyr Asp Ala Asp Val Ile Glu Tyr Val Gly Asp Phe Ile
385                 390                 395                 400

AAA CCT ATT AAT AAA CCA ATG TAT AAC ATT TAT AGA ACA CTT AAA AAA 1248
Lys Pro Ile Asn Lys Pro Met Tyr Asn Ile Tyr Arg Thr Leu Lys Lys
                405                 410                 415

CTA AAG AAA 1257
Leu Lys Lys
```

The synthesis of the FemA protein of the present invention may proceed by solid phase peptide synthesis or by recombinant methods. Both methods are described in U.S. Patent No. 4,617,149, the entire teaching of which is herein incorporated by reference. Recombinant methods are preferred if a high yield is desired. The principles of solid phase chemical synthesis of polypeptides are well known in the art and may be found in general texts in the area such as Dugas, H. and Penney, C., *Bioorganic Chemistry* (1981), Springer-Verlag, New York, pgs. 54-92.

Synthesis of the FemA protein can be achieved by recombinant DNA technology. Synthetic genes, the *in vitro* or *in vivo* transcription and translation of which will result in the production of the FemA protein may be constructed by techniques well known in the art. Owing to the natural degeneracy of the genetic code, the skilled artisan will recognize that a sizable yet definite number of DNA sequences may be constructed which encode the FemA protein. All such genes are provided by the present invention. A preferred gene encoding the FemA protein is the natural *femA* gene of *Staphylococcus epidermidis,* which is SEQ ID NO: 1. This preferred *femA* gene is available on an ~ 3.7 kb *EcoRI* restriction fragment of plasmid pPSJ180, publicly available and on deposit in *Escherichia coli* DH5α at the National Center for Agricultural Utilization Research, 1815 North University Street, Peoria, Illinois 61604-39999, under accession number NRRL B-21024 (date of deposit: December 8, 1992). A restriction site and function map of pPSJ180 is provided in Figure 1 of the drawings.

The *femA* gene may be created by synthetic methodology. Such methodology of synthetic-gene construction is well known in the art. *See* Brown et al. (1979) *Methods in Enzymology,* Academic Press, N.Y., **68**:109-151. The *femA* DNA sequence may be generated using a conventional DNA synthesizing apparatus such as the Applied Biosystems Model 380A or 380B DNA synthesizers (commercially available from Applied Biosystems, Inc., 850 Lincoln Center Drive, Foster City, CA 94404).

To effect the translation of the FemA protein, one inserts the engineered synthetic DNA sequence in any of a large number of appropriate recombinant DNA expression vectors through the use of appropriate restriction endonucleases and DNA ligase. The synthetic *femA* gene should be designed to possess restriction endonuclease cleavage sites at either end of the transcript to facilitate isolation from and integration into these amplification and expression plasmids. The particular endonucleases employed will be dictated by the restriction endonuclease cleavage pattern of the parent expression vector to be employed. The choice of restriction sites are chosen so as to properly orient the FemA coding sequence with control sequences to achieve proper in-frame reading and expression of the FemA molecule. The FemA coding sequence must be positioned so as to be in proper reading frame with the promoter and ribosome binding site of the expression vector, both of which are functional in the host cell in which the FemA protein is to be expressed. The FemA protein may be expressed in any number of well-known eucaryotic or procaryotic hosts using known promoters and vectors. Some of the potential hosts, in addition to *E. coli,* include the yeasts *Saccharomyces cerevisiae* and *Pichia pastoris, Bacillus,* and cells infected with baculovirus.

To achieve efficient transcription of the synthetic gene, said gene must be operably associated with a promoter operator region. In one practice of the invention, the promoter-operator region of the synthetic gene encoding SEQ ID NO: 2 is placed in the same sequential orientation with respect to the ATG start codon of the synthetic gene as the promoter-operator occupies with respect to the ATG-start codon of the gene from which it was derived. Synthetic or modified promoter operator regions have been created and are well known in the art. When employing such synthetic or modified promoter-operator regions they should be oriented with respect to the ATG-start codon of the *femA* gene as directed by their creators. In one practice of the invention as exemplified herein, where the host cell is an *E. Coli* host cell, said promoter-operator region is the phage T7 promoter-operator region.

A variety of expression vectors useful for transforming procaryotic cells are well known in the art. A preferred vector for expression in an *E. coli* host cell is derived from *E. coli* plasmid pET-11A, which comprises the phage T7 promoter. A restriction site and function map of pET-11A appears in Figure 2 of the accompanying drawings. Plasmid pET-11A is publicly available from Novagen, Inc. (565 Science Drive, Madison, WI 53711) under catalog #69436-1. The preferred host strain is *E. coli* BL21(DE3), also available from Novagen under catalog #69387-1.

The techniques of transforming cells with the aforementioned vectors are well known in the art and may be found in such general references as Sambrook et al., *Molecular Cloning: A Laboratory Manual* (1988), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY or Ausubel et al., *Current Protocols in Molecular Biology* (1989), John Wiley & Sons, New York, NY and supplements. The techniques involved in the transformation of *E. coli* cells used in the preferred practice of the invention as exemplified herein are well known in the art. The precise conditions under which the transformed *E. coli* cells are cultured is dependent on the nature of the *E. coli* host cell line and the expression or cloning vectors employed. For example, vectors which incorporate thermoinducible promoter-operator regions, such as the cI857 thermoinducible lambda-phage promoter-operator region, require a temperature shift from about 30 to about 40°C. in the culture conditions so as to induce

protein synthesis.

In a preferred embodiment of the invention *E. coli* K12 BL21 (DE3) cells were employed as host cells but numerous other cell lines are available. The transformed host cells are then plated on appropriate media under the selective pressure of the antibiotic corresponding to the resistance gene present on the expression plasmid. The cultures are then incubated for a time and temperature appropriate to the host cell line employed. Specifically, with *E. coli* K12 BL21 (DE3) cells, the *femA* gene is placed under the control of a promoter transcribed specifically by the T7 RNA polymerase. Induction of transcription of the *femA* gene is accomplished by the addition of isopropylthiogalactoside (IPTG) to the growth medium, which induces expression of the T7 RNA polymerase gene under the control of the *lacUV5* promoter. The T7 RNA polymerase is then available to transcribe the *femA* gene.

General techniques of protein purification are well-known to those of ordinary skill in the art. *See* Creighton, T.E., *Protein Structure: A Practical Approach* (1989), IRL Press, Oxford, England and Bollag, D.M. and Edelstein, S.J., *Protein Methods* (1991), Wiley-Liss, New York, NY. Proteins which are expressed in high-level bacterial expression systems characteristically aggregate in granules or inclusion bodies which contain high levels of the overexpressed protein. Kreuger et al. (1990) in *Protein Folding,* Gierasch and King, eds., pgs 136-142, American Association for the Advancement of Science Publication No. 89-18S, Washington, D.C. and Sambrook et al., *Molecular Cloning: A Laboratory Manual* (1988), pp. 17.37-17.41, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. The FemA protein sometimes aggregates into inclusion bodies when expressed under the control of phage T7 promoter. Such protein aggregates must be solubilized to provide further purification and isolation of the desired protein product. A variety of techniques using strongly denaturing solutions such as guanidinium-HCl and/or weakly denaturing solutions such as dithiothreitol (DTT) are used to solubilize the proteins.

Recombinantly produced proteins may be purified by a variety of techniques well known in the art such as ion exchange chromatography, size exclusion chromatography, electrophoresis, differential centrifugation, reversed phase high performance liquid chromatography, immunoaffinity chromatography, and the like. Protocols for use of these individual techniques or combinations thereof are well known in the art. Gradual removal of the denaturing agents (often by dialysis) in a refolding solution allows the denatured protein to assume its native conformation. The particular conditions for denaturation and refolding are determined by the particular protein expression system and/or protein in question. The S-sulfonates of the peptide molecules are converted to the disulfide paired, folded FemA molecules using a combination of high pH and added thiol in substantial accordance with the teaching of Frank, B.H. et al., (1981) in *Peptides. Synthesis, Structure and Function. Proceedings of the Seventh American Peptide Symposium* (Rich, D.H. and Gross, E., eds.) pp. 729-738, Pierce Chemical Co., Rockford, IL.

The *femA* gene may be used in gene disruption studies in *Staphylococcus epidermidis.* Although it is believed that the FemA protein is involved in the formation of a pentaglycine bridge in the cell wall of the bacterium, gene disruption will allow one to ascertain the precise effect of the loss of the *femA* gene. Gene disruption experiments in *Staphylococcus aureus* have revealed that a loss of *femA* results in an ~40% reduction in cell wall glycine content. A similar result might be anticipated for *S. epidermidis*. Once determined, this information can be used to generate an assay for agents which inhibit the FemA protein, and are therefore useful in combination with antibiotics to treat methicillin-resistant bacteria.

The FemA protein of SEQ ID NO: 2 may be produced by recombinant methods. Recombinant methods are preferred if a high yield is desired. The present invention thus comprises a method for constructing a recombinant host cell capable of expressing SEQ ID NO: 2, said method comprising transforming a host cell with a recombinant DNA vector that comprises an isolated DNA sequence of Claim 1. The present invention also comprises a method for expressing SEQ ID NO: 2 in a recombinant host cell; said method comprising culturing said transformed host cell of Claim 5 under conditions suitable for gene expression.

The following Examples are provided to further illustrate and exemplify, but not limit the scope of, the invention.

## Example 1

### Source of the *Staphylococcus epidermidis femA* gene

### Isolation of Plasmid pPSJ180

A lyophil of *E. coli* K12 DH5α/pPSJ180 can be obtained from the Northern Regional Research Laboratories (NRRL), Peoria, Illinois 61604, under the accession number NRRL B-21024 (date of deposit: December 8, 1992). The pPSJ180 plasmid may be isolated from *E. coli* K12 DH5α/pPSJ180 using techniques well-known

to those skilled in the art. *See* Sambrook et al., *Molecular Cloning: A Laboratory Manual* (1988), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY or Ausubel et al., *Current Protocols in Molecular Biology* (1989), John Wiley & Sons, New York, NY and supplements.

Isolation of the *Staphylococcus epidermidis femA* gene via the polymerase chain reaction

Isolated plasmid pPSJ180 is used as the template for the polymerase chain reaction at a concentration of 10 ng/reaction. $Vent_R$™ DNA polymerase (2 units/μl, Catalog #254, New England Biolabs, 32 Tozer Road, Beverly, MA 01915-9965) is used with standard $Vent_R$™ DNA polymerase buffer (1X= 10 mM KCl, 20 mM Tris-HCl (pH 8.8 at 25°C), 10 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$, 0.1% Triton X-100). The PCR primers used are AGATATAAAGATCTAGATGGGAGTTATGAA (SEQ ID NO: 3) and ATTTCATAATTAGATGGATCCCTTCTTAAAATC (SEQ ID NO: 4). The reaction is carried out by 3 cycles of 94°C for 15 seconds, 40°C for 15 seconds and 72°C for 1 minute followed by 20 cycles of 94°C for 15 seconds, 55°C for 15 seconds and 72°C for 1 minute.

The reaction is transferred to a Centricon 100 microconcentrator (Amicon, Inc., 72 Cherry Hill, Beverly, MA 01915) and washed with 1 ml of water. The microconcentrator is then subjected to centrifugation at 3000 rpm in a microcentrifuge for 30 minutes. The reaction is then diluted to 200 μl (from ~50 μl) with 1X *XbaI* restriction enzyme buffer. To this is added 50 units of *XbaI* and 50 units of *BamHI.* The DNA is then digested for 90 minutes at 37°C. The DNA is phenol extracted and ethanol precipitated.

Example 2

Construction of an Expression Plasmid Containing the *femA* Gene

The DNA created in Example 1 is then ligated to the 5.6 kb *XbaI-BamHI* fragment of pET-11A (available from Novagen, Inc. (565 Science Drive, Madison, WI 53711) under catalog #69436-1. This plasmid is then transformed into *E. coli* BL21 (DE3) (also available from Novagen, Inc. under catalog #69387-1) using techniques well known to those of ordinary skill in the art.

Example 3

Expression of the FemA Protein

*E.* coli BL21 (DE3) transformed with the *femA* expression plasmid are grown overnight in TY broth (per liter 10 g tryptone, 5 g yeast extract and 5 g NaCl) and 100 μg/ml ampicillin. The cells are then diluted 1/50 into TY broth + ampicillin and grown at 37°C for 60 minutes. Expression is induced by adding ispropylthiogalactoside (IPTG) to 0.4 mM. Samples are taken at 0 and 6 hours and run on a % SDS-polyacrylamide gel using techniques described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* (1988), pp. 18.47-18.59, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. An induced protein band is visible by staining with Coomassie Blue at the predicted size of 49,000 daltons.

# SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: ELI LILLY AND COMPANY
(B) STREET: Lilly Corporate Center
(C) CITY: Indianapolis
(D) STATE: Indiana
(E) COUNTRY: United States of America
(F) ZIP: 46285

(ii) TITLE OF INVENTION: FEMA GENE OF STAPHYLOCOCCUS EPIDERMIDIS, FEMA PROTEIN, AND VECTORS AND MICROORGANISMS COMPRISING THE FEMA GENE

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: C. M. Hudson
(B) STREET: Erl Wood Manor
(C) CITY: Windlesham
(D) STATE: Surrey
(E) COUNTRY: United Kingdom
(F) ZIP: GU20 6PH

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: Macintosh
(C) OPERATING SYSTEM: Macintosh 7.0
(D) SOFTWARE: Microsoft Word 5.1

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1257 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1257

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATG AAG ATG AAG TTT ACG AAT TTG ACA GCT AAA GAA TTT AGT GAC TTT 48
Met Lys Met Lys Phe Thr Asn Leu Thr Ala Lys Glu Phe Ser Asp Phe
1               5                  10                  15
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ACT GAT CGT ATG ACA TAT AGT CAT TTT ACA CAA ATG GAA GGT AAT TAC 96
Thr Asp Arg Met Thr Tyr Ser His Phe Thr Gln Met Glu Gly Asn Tyr
            20                  25                  30

GAA TTA AAG GTT GCT GAA GGT ACC GAG TCA CAT TTA GTT GGA ATT AAA 144
Glu Leu Lys Val Ala Glu Gly Thr Glu Ser His Leu Val Gly Ile Lys
        35                  40                  45

AAT AAT GAT AAC GAA GTG ATT GCA GCT TGT TTA TTA ACA GCT GTT CCT 192
Asn Asn Asp Asn Glu Val Ile Ala Ala Cys Leu Leu Thr Ala Val Pro
    50                  55                  60

GTA ATG AAA ATA TTT AAA TAT TTT TAT TCC AAT CGC GGT CCA GTA ATA 240
Val Met Lys Ile Phe Lys Tyr Phe Tyr Ser Asn Arg Gly Pro Val Ile
65                  70                  75                  80

GAT TAT AAT AAT AAA GAG CTT GTA CAT TTT TTC TTT AAT GAA TTG AGT 288
Asp Tyr Asn Asn Lys Glu Leu Val His Phe Phe Phe Asn Glu Leu Ser
                85                  90                  95

AAA TAT GTA AAA AAA TAT AAT TGT TTA TAT TTA AGA GTT GAC CCA TAC 336
Lys Tyr Val Lys Lys Tyr Asn Cys Leu Tyr Leu Arg Val Asp Pro Tyr
            100                 105                 110

CTT CCA TAT CAA TAT TTA AAT CAT GAG GGA GAA ATA ACT GGA AAT GCA 384
Leu Pro Tyr Gln Tyr Leu Asn His Glu Gly Glu Ile Thr Gly Asn Ala
        115                 120                 125

GGT CAT GAT TGG ATT TTT GAT GAA TTA GAG AGT TTA GGA TAT AAA CAC 432
Gly His Asp Trp Ile Phe Asp Glu Leu Glu Ser Leu Gly Tyr Lys His
    130                 135                 140

GAA GGA TTC CAC AAA GGA TTT GAT CCT GTA TTA CAA ATC CGA TAT CAT 480
Glu Gly Phe His Lys Gly Phe Asp Pro Val Leu Gln Ile Arg Tyr His
145                 150                 155                 160

TCT GTT CTA AAT TTA GCA AAC AAA AGT GCT AAT GAT GTT TTA AAA AAC 528
Ser Val Leu Asn Leu Ala Asn Lys Ser Ala Asn Asp Val Leu Lys Asn
                165                 170                 175

ATG GAT GGT TTA AGA AAG CGT AAT ACT AAA AAA GTT AAG AAA AAT GGA 576
Met Asp Gly Leu Arg Lys Arg Asn Thr Lys Lys Val Lys Lys Asn Gly
            180                 185                 190

GTT AAA GTC CGC TTT TTA TCT GAA GAA GAG TTA CCT ATA TTT AGG TCA 624
Val Lys Val Arg Phe Leu Ser Glu Glu Glu Leu Pro Ile Phe Arg Ser
        195                 200                 205

TTT ATG GAG GAT ACC TCT GAA ACT AAA GAT TTT GCA GAT AGA GAA GAT 672
Phe Met Glu Asp Thr Ser Glu Thr Lys Asp Phe Ala Asp Arg Glu Asp
    210                 215                 220

AGT TTT TAT TAC AAC AGA TTC AAA CAT TAT AAA GAC CGT GTT TTA GTA 720
Ser Phe Tyr Tyr Asn Arg Phe Lys His Tyr Lys Asp Arg Val Leu Val
225                 230                 235                 240
```

```
CCA CTA GCC TAT ATT AAC TTT GAT GAG TAT ATA GAG GAA CTA AAT AAT 768
Pro Leu Ala Tyr Ile Asn Phe Asp Glu Tyr Ile Glu Glu Leu Asn Asn
                245                 250                 255

GAA AGA AAT GTG CTT AAT AAA GAT TAT AAT AAA GCT TTA AAA GAC ATT 816
Glu Arg Asn Val Leu Asn Lys Asp Tyr Asn Lys Ala Leu Lys Asp Ile
            260                 265                 270

GAG AAA CGT CCA GAG AAT AAA AAA GCA CAT AAC AAA AAG GAA AAT TTA 864
Glu Lys Arg Pro Glu Asn Lys Lys Ala His Asn Lys Lys Glu Asn Leu
        275                 280                 285

GAA CAA CAA CTC GAT GCA AAT CAG CAA AAA ATT AAT GAA GCT AAA AAC 912
Glu Gln Gln Leu Asp Ala Asn Gln Gln Lys Ile Asn Glu Ala Lys Asn
    290                 295                 300

TTA AAA CAA GAA CAT GGC AAT GAA TTA CCC ATC TCT GCT GGC TTC TTT 960
Leu Lys Gln Glu His Gly Asn Glu Leu Pro Ile Ser Ala Gly Phe Phe
305                 310                 315                 320

ATA ATT AAT CCG TTT GAA GTA GTT TAC TAC GCT GGT GGA ACT TCA AAT 1008
Ile Ile Asn Pro Phe Glu Val Val Tyr Tyr Ala Gly Gly Thr Ser Asn
                325                 330                 335

CGT TAT CGC CAT TTT GCA GGG AGC TAT GCG GTT CAA TGG AAG ATG ATT 1056
Arg Tyr Arg His Phe Ala Gly Ser Tyr Ala Val Gln Trp Lys Met Ile
            340                 345                 350

AAC TAT GCA ATT GAA CAT GGT ATT AAT CGG TAT AAT TTC TAT GGT ATT 1104
Asn Tyr Ala Ile Glu His Gly Ile Asn Arg Tyr Asn Phe Tyr Gly Ile
        355                 360                 365

AGT GGT GAC TTT AGT GAA GAT GCT GAA GAT GCT GGC GTA GTT AAG TTT 1152
Ser Gly Asp Phe Ser Glu Asp Ala Glu Asp Ala Gly Val Val Lys Phe
    370                 375                 380

AAA AAG GGC TAT GAT GCC GAT GTT ATA GAA TAC GTT GGT GAC TTT ATT 1200
Lys Lys Gly Tyr Asp Ala Asp Val Ile Glu Tyr Val Gly Asp Phe Ile
385                 390                 395                 400

AAA CCT ATT AAT AAA CCA ATG TAT AAC ATT TAT AGA ACA CTT AAA AAA 1248
Lys Pro Ile Asn Lys Pro Met Tyr Asn Ile Tyr Arg Thr Leu Lys Lys
                405                 410                 415

CTA AAG AAA 1257
Leu Lys Lys
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 419 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Lys Met Lys Phe Thr Asn Leu Thr Ala Lys Glu Phe Ser Asp Phe
1               5                  10                  15

Thr Asp Arg Met Thr Tyr Ser His Phe Thr Gln Met Glu Gly Asn Tyr
            20                  25                  30

Glu Leu Lys Val Ala Glu Gly Thr Glu Ser His Leu Val Gly Ile Lys
        35                  40                  45

Asn Asn Asp Asn Glu Val Ile Ala Ala Cys Leu Leu Thr Ala Val Pro
    50                  55                  60

Val Met Lys Ile Phe Lys Tyr Phe Tyr Ser Asn Arg Gly Pro Val Ile
65                  70                  75                  80

Asp Tyr Asn Asn Lys Glu Leu Val His Phe Phe Phe Asn Glu Leu Ser
                85                  90                  95

Lys Tyr Val Lys Lys Tyr Asn Cys Leu Tyr Leu Arg Val Asp Pro Tyr
            100                 105                 110

Leu Pro Tyr Gln Tyr Leu Asn His Glu Gly Glu Ile Thr Gly Asn Ala
        115                 120                 125

Gly His Asp Trp Ile Phe Asp Glu Leu Glu Ser Leu Gly Tyr Lys His
    130                 135                 140

Glu Gly Phe His Lys Gly Phe Asp Pro Val Leu Gln Ile Arg Tyr His
145                 150                 155                 160

Ser Val Leu Asn Leu Ala Asn Lys Ser Ala Asn Asp Val Leu Lys Asn
                165                 170                 175

Met Asp Gly Leu Arg Lys Arg Asn Thr Lys Lys Val Lys Lys Asn Gly
            180                 185                 190

Val Lys Val Arg Phe Leu Ser Glu Glu Glu Leu Pro Ile Phe Arg Ser
        195                 200                 205
```

```
Phe Met Glu Asp Thr Ser Glu Thr Lys Asp Phe Ala Asp Arg Glu Asp
    210                 215                 220

Ser Phe Tyr Tyr Asn Arg Phe Lys His Tyr Lys Asp Arg Val Leu Val
225                 230                 235                 240

Pro Leu Ala Tyr Ile Asn Phe Asp Glu Tyr Ile Glu Glu Leu Asn Asn
                245                 250                 255

Glu Arg Asn Val Leu Asn Lys Asp Tyr Asn Lys Ala Leu Lys Asp Ile
            260                 265                 270

Glu Lys Arg Pro Glu Asn Lys Lys Ala His Asn Lys Lys Glu Asn Leu
        275                 280                 285

Glu Gln Gln Leu Asp Ala Asn Gln Gln Lys Ile Asn Glu Ala Lys Asn
    290                 295                 300

Leu Lys Gln Glu His Gly Asn Glu Leu Pro Ile Ser Ala Gly Phe Phe
305                 310                 315                 320

Ile Ile Asn Pro Phe Glu Val Val Tyr Tyr Ala Gly Gly Thr Ser Asn
                325                 330                 335

Arg Tyr Arg His Phe Ala Gly Ser Tyr Ala Val Gln Trp Lys Met Ile
            340                 345                 350

Asn Tyr Ala Ile Glu His Gly Ile Asn Arg Tyr Asn Phe Tyr Gly Ile
        355                 360                 365

Ser Gly Asp Phe Ser Glu Asp Ala Glu Asp Ala Gly Val Val Lys Phe
    370                 375                 380
Lys Lys Gly Tyr Asp Ala Asp Val Ile Glu Tyr Val Gly Asp Phe Ile
385                 390                 395                 400

Lys Pro Ile Asn Lys Pro Met Tyr Asn Ile Tyr Arg Thr Leu Lys Lys
                405                 410                 415

Leu Lys Lys
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGATATAAAG ATCTAGATGG GAGTTATGAA 30

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATTTCATAAT TAGATGGATC CCTTCTTAAA ATC 33

**Claims**

1. A DNA compound that comprises an isolated DNA sequence encoding SEQ ID NO: 2.
2. The DNA compound of Claim 1 which comprises the isolated DNA sequence which is SEQ ID NO: 1.
3. A recombinant DNA vector that comprises the isolated DNA sequence of Claim 1.
4. A recombinant DNA vector of Claim 3 that further comprises a promoter positioned to drive expression of said isolated DNA sequence.
5. A method for constructing a recombinant host cell capable of expressing SEQ ID NO: 2, said method comprising transforming a host cell with a recombinant DNA vector that comprises an isolated DNA sequence of Claim 1.
6. A method for expressing SEQ ID NO: 2 in a recombinant host cell; said method comprising culturing said transformed host cell of Claim 5 under conditions suitable for gene expression.
7. A recombinant host cell transformed with a recombinant DNA vector of Claim 3.

8. The protein, in purified form, encoded by SEQ ID NO:2.

FIG. 1

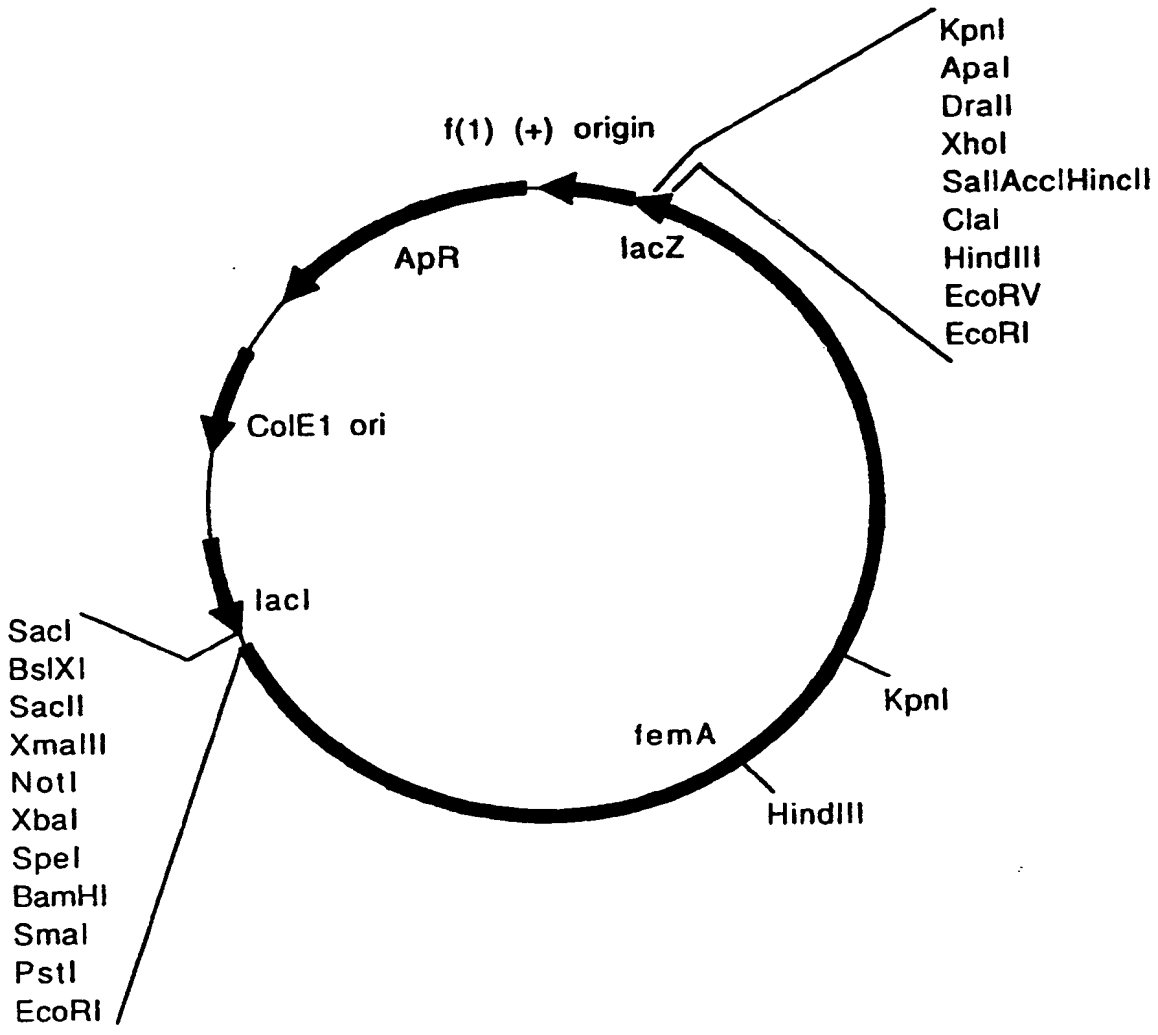
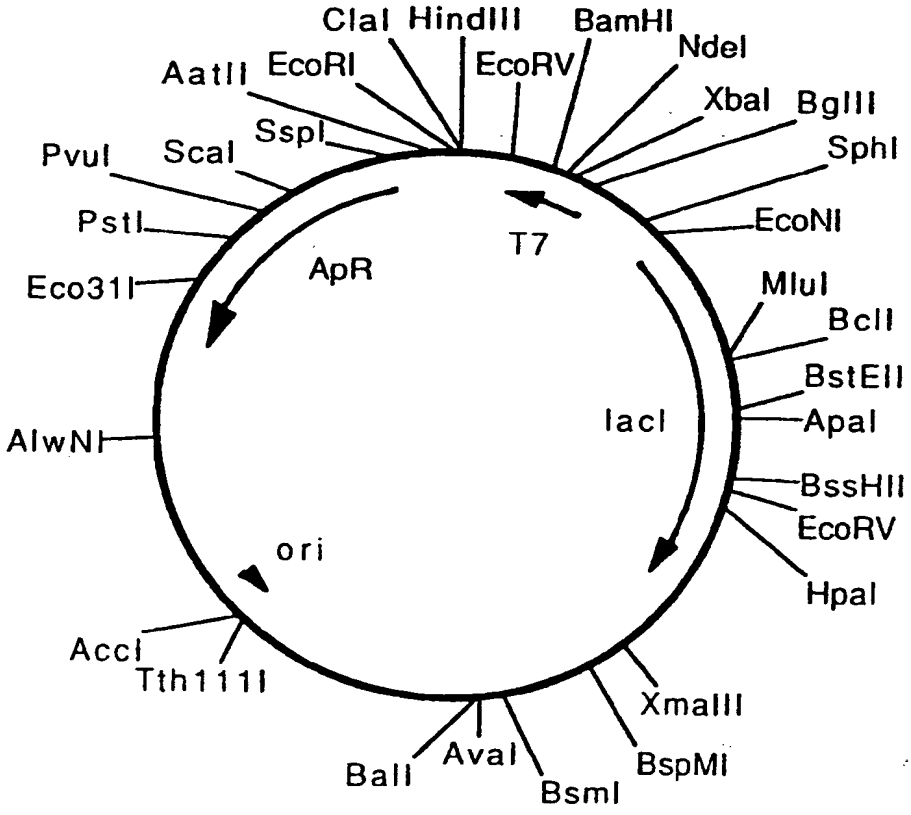


FIG. 2



(19) Europäisches Patentamt
European Patent Office
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(84) Designated Contracting States : **AT BE CH DE DK ES FR GB GR IE IT LI LU NL PT SE**

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**Lilly Industries Limited**
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**Windlesham Surrey GU20 6PH (GB)**
**Declaration under Rule 28(4) EPC (expert solution)**

## (54) Fem A gene of staphylococcus epidermidis, fem A protein, and vectors of microorganisms comprising the fem A gene.

(57) The instant invention provides the *femA* gene of *Staphylococcus epidermidis* and all degenerate sequences thereof, the protein encoded by the *femA* gene (FemA), and vectors and microorganisms comprising genes encoding the FemA protein.

EP 0 625 575 A3

European Patent Office

# EUROPEAN SEARCH REPORT

Application Number
EP 94 30 2950

## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int.Cl.5) |
|---|---|---|---|
| Y | MOL. GEN. GENET., vol.219, 1989 pages 263 - 269 BERGER-BÄCHI, B. ET AL. 'FemA, a host-mediated factor essential for methicillin resistance in Staphylococcus aureus: Molecular cloning and characterization' see Table 1 and Figure 4 --- | 1-8 | C12N15/31 C12P21/02 C07K13/00 |
| Y | ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol.36, no.12, December 1992 pages 2617 - 2621 HÜRLIMANN-DALEL, R. ET AL. 'Survey of the methicillin resistance-associated genes mecA, mecR1-mecI, and femA-femB in clinical isolates of methicillin -resistant Staphylococcus aureus' see Figure 1 --- | 1-8 | |
| A | WO-A-91 08305 (U-GENE RESEARCH) 13 June 1991 see pages 1-4 ----- | 1 | TECHNICAL FIELDS SEARCHED (Int.Cl.5) C07K |

The present search report has been drawn up for all claims

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| MUNICH | 15 December 1994 | Alt, G |

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)